Factors Affecting the Determination of Available Lysine in Whey with 2,4,6-Trinitrobenzene Sulfonic Acid

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Abstract

Lactose in cheese whey interferes with the determination of biologically available lysine in whey proteins when the reagent 2,4,6-trinitrobenzene sulfonic acid is employed. Loss of the epsilon-TNP-derivative of lysine is dependent upon the amount of lactose in the test material. The sample weight chosen for analysis also influences results with this reagent. The larger the sample of either pure protein or carbohydrate containing material, the smaller the amount of available lysine found. By careful selection of experimental conditions and by application of a correction formula for lactose interference, the available lysine in whey protein can be rapidly approximated with the 2,4,6-trinitrobenzene sulfonic acid method. Results compare favorably with those obtained by an established method.

Introduction

Economical salvage of cheese whey, an underutilized source of high grade animal protein, has provided a challenge for considerable research. In our attempts to develop methods for the fractionation and dehydration of cheese whey, we became concerned about the possible loss in whey nutrients, particularly lysine, during processing.

The complete destruction of lysine in processed foods through the browning reaction or its conversion into biologically unavailable forms has been studied extensively (8, 10). However, chemical methods for the determination of biologically available lysine in foods remained complex and time consuming (2, 4, 11, 12). Accordingly, we were interested in a procedure described recently by Kakade and Liener (9) in which 2,4,6-trinitrobenzene sulfonic acid (TNBS) was used for the routine assay of available lysine in numerous samples of isolated proteins and dehydrated foods.

Our present paper describes the corrections and modifications necessary to improve the accuracy of the results when TNBS is used to determine available lysine in whey based products.

Materials and Methods[1]

Whey proteins used to construct a model system were obtained as follows: Acid whey was obtained as a by-product from a commercial Cottage cheese manufacturing operation in the Washington, D. C. area. This whey was concentrated to 50% total solids, exhaustively dialyzed against distilled water at 4 C and lyophilized before analysis.

Samples of roller and spray dried sweet wheys and lactalbumin were obtained from plants in the Midwest.

Bovine serum albumin was purchased from Mann Research Laboratories, New York City, and β-lactoglobulin was prepared by the method of Fox et al. (6).

Epsilon-trinitrophenyl (TNP)-L-lysine hydrochloride and 2,4-dinitrofluorobenzene (DNFB) were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. The sodium salt of picryl sulfonic acid was a product of Aldrich Chemical Co., Milwaukee, Wis., and lactose powder and reagent grade hydrochloric acid were bought from J. T. Baker Chemical Co., Phillipsburg, N. J.

Nitrogen analyses were made by a standard micro-Kjeldahl procedure (1). Lactose was determined by the method of Fox et al. (5). Total lysine in the samples was determined with a Beckman Model 120 C amino acid analyzer in accordance with the method of Spackman et al. (13). Aliquots of each sample suspended in 6 *N* HCl were sealed under vacuum in Pyrex tubes and hydrolyzed at 110 C. Individual aliquots were hydrolyzed for 22, 46, and 70 hr to ascertain the maximum amount of lysine liberated.

Available lysine was measured directly according to the procedure of Kakade and

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[1] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Liener (9). Variations in this procedure which we consistently used included reduction in sample size and amount of reagent. In routine analyses, sample weights were adjusted to yield .2 mg of protein per test. The amount of TNBS reagent was reduced to .05% due to the high absorbance it imparted to the blanks at 346 mμ. Blanks as well as samples were run in triplicate. Blanks for the standard curve were reagent blanks. All blanks for the samples contained the protein sample. In experiments where lactose was added to the standard solutions or samples, equivalent amounts of lactose were added to blanks. All absorbances were measured on a Hitachi Model 101 spectrophotometer. Filtrations were performed with Whatman filter papers (numbers 2, 12, 42, 50).

"Blocked" lysine was measured as described by Blom et al. (3) without addition of dinitrophenol before hydrolysis. The quantity of lysine which was blocked against reaction with DNFB was measured on the amino acid analyzer under the same operating conditions as measure total lysine. The difference between the blocked and total values is considered to be the available lysine.

Results

In the analysis of protein foodstuffs, Kakade and Liener (9) suggest that filtration of the derivative containing solution after digestion may be necessary to remove insoluble particulate matter. Figure 1 has two standard curves, one constructed from data obtained by carrying varying amounts of E-TNP-L-lysine through the procedure as recommended by Kakade and Liener (9) and the other by including a filtration. Blanks for the filtered standard curve are also filtered. From this figure it is obvious that derivative is lost during filtration and must be corrected for if filtration is not routinely used during standard curve construction and analyses of samples. The four types of Whatman filter papers yielded identical results.

The magnitude of the correction necessary with a standard curve constructed from data with unfiltered solutions to determine available lysine in samples that require filtration is shown in Figure 2. These data describe a typical binding curve. Consequently the necessary correction becomes large when the amount of colored derivative in the solution filtered is small.

Derivative yield is influenced not only by filtration but also by lactose in the system. This is shown in Figure 3 wherein four standard curves are constructed with E-TNP-L-lysine with the addition of varying lactose. From this figure, it can be seen that increasing lactose results in increased destruction of the E-TNP-L-lysine derivative. The maximum absorbance of the derivative is not shifted from 346 mμ in the presence of hydrolyzed lactose.

The extent of this destruction of derivative in a model system containing all the whey proteins and varying amounts of lactose is shown in Figure 4. The points are results obtained with the TNBS procedure to determine avail-

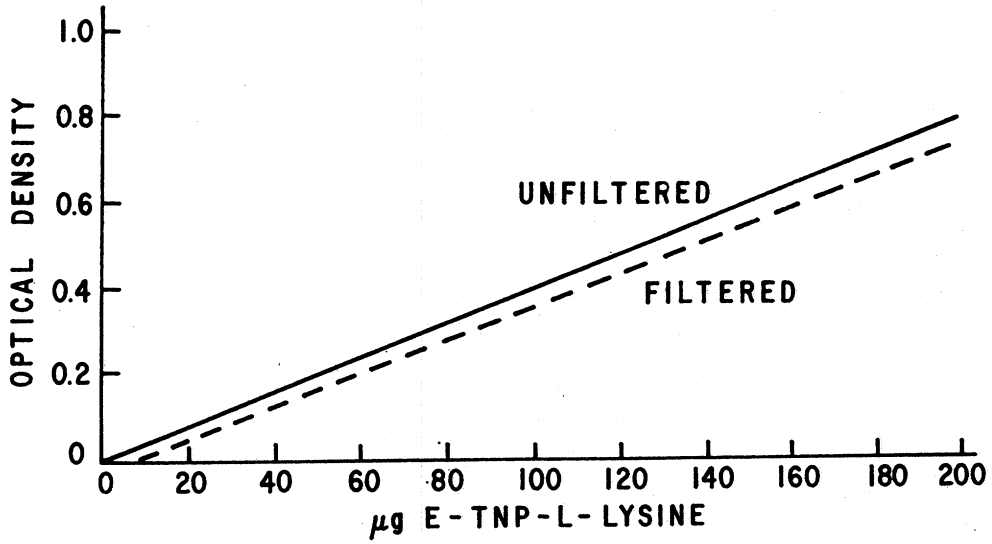

FIG. 1. Effect of filtration on the standard curve.

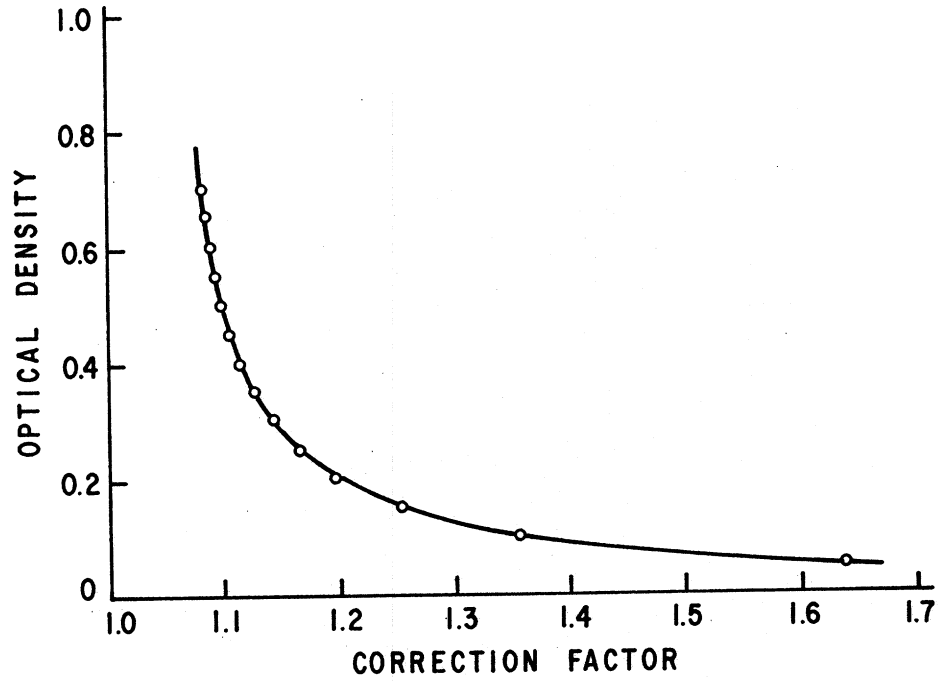

FIG. 2. Correction curve for loss of derivative due to filtration. The correction factor is the ratio of the micrograms of E-TNP-L-lysine from the filtered standard curve to the micrograms of E-TNP-L-lysine from the unfiltered curve.

able lysine in solutions containing .5 mg whey protein and increasing amounts of lactose. The curve is exponential. Additional data obtained in similar fashion are shown in Figure 5 as a log-log plot. Least squares analysis showed the data represent a straight line described by the equation:

$$\log Y = -1.4936 + 0.9057 \log X$$

where Y = % E-TNP-L-lysine derivative lost and X = concentration of lactose in the sam-

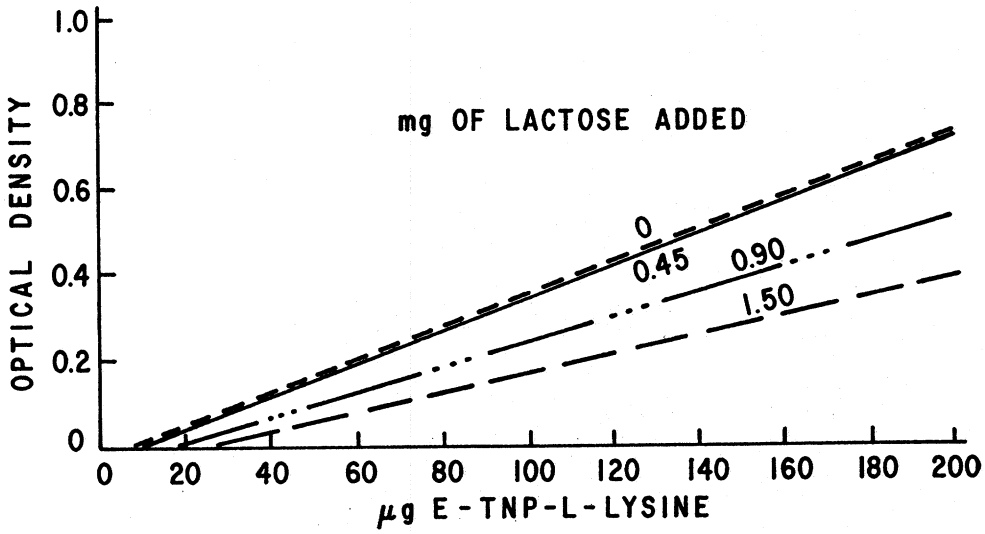

FIG. 3. Effect of added lactose on the filtered standard curve.

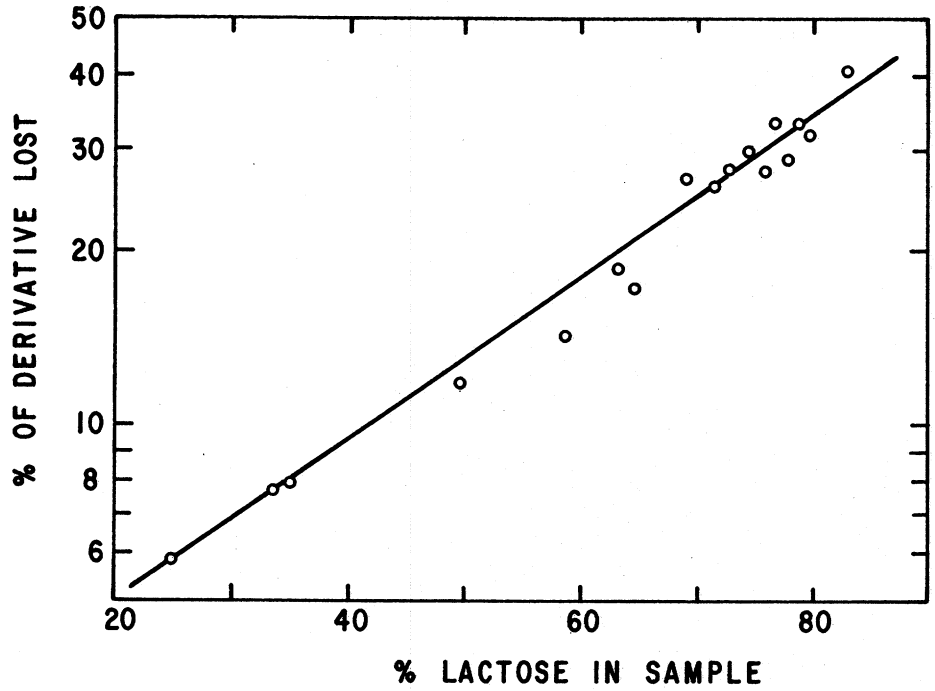

FIG. 4. Effect of added lactose on a model whey protein mixture containing .5 mg protein. The method of averages analysis of the data yield a straight line described by log Y = .225 + .0167 X.

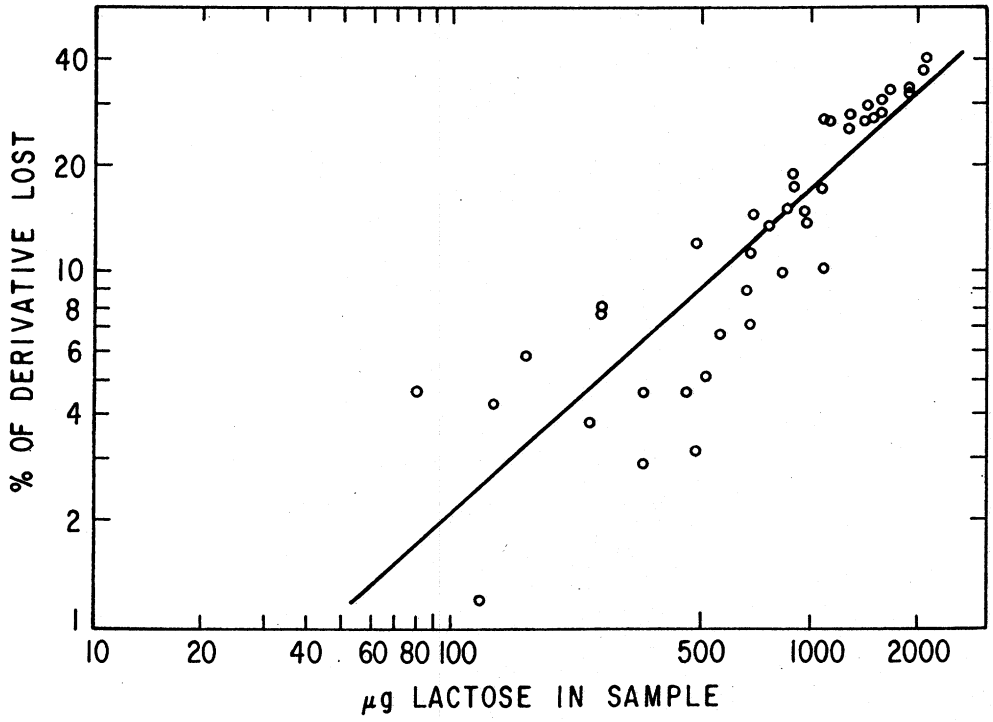

FIG. 5. Effect of added lactose on model whey protein mixtures of three different sample sizes.

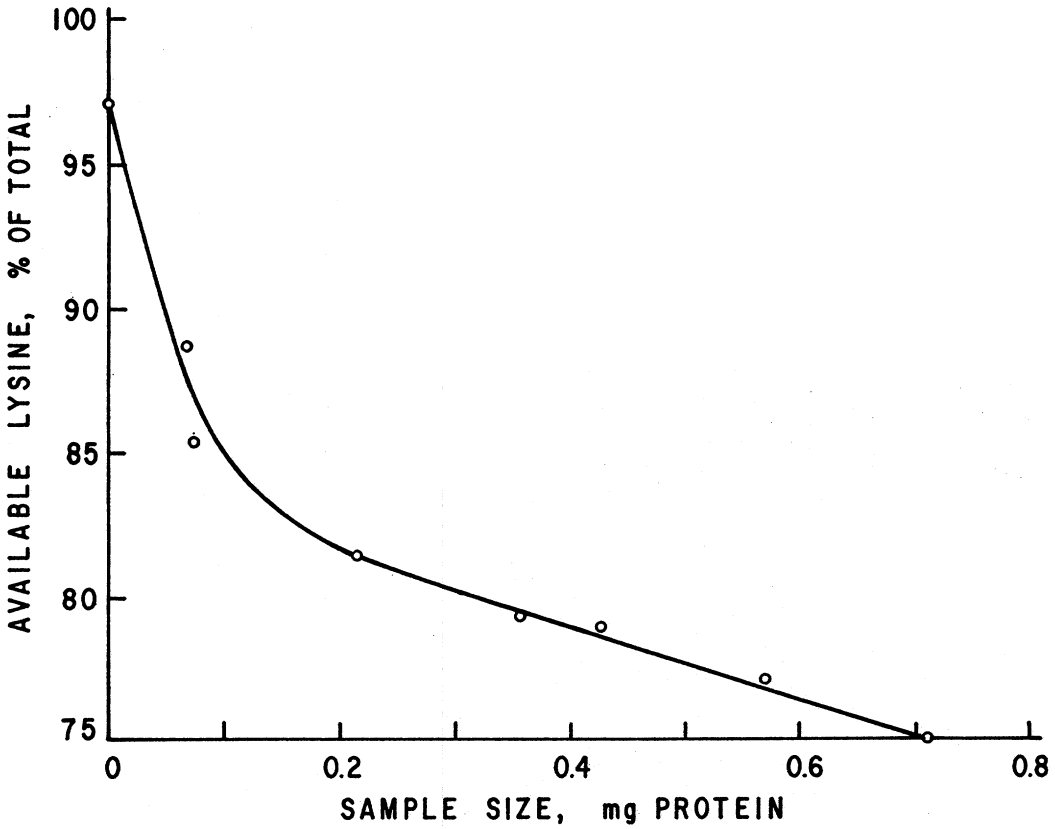

FIG. 6. Effect of sample size on the amount of available lysine found in bovine serum albumin. The O sample size value is the amount of available lysine found by the blocked method.

ple. The correlation coefficient is .880. We employ this equation to correct our data for lactose interference because it is useful over a range of protein sample sizes. The observed scatter in Figure 5 was attributable to variation in protein sample size of from .2 to .5 mg. Figure 6 demonstrates the effect of protein sample size on the available lysine of bovine serum albumin using the TNBS procedure. Increasing the amount of reagent did not overcome this effect. Similar results were obtained with β-lactoglobulin and whey protein containing products. Decreases in available lysine in these materials were not as large as for bovine serum albumin for protein sample sizes below .4 mg.

If the protein sample size is standardized, if allowance for loss of derivative due to filtration is made, and if the values are corrected for lactose in the product, concurrence with data procured by a more established method can be observed. This is shown in Table 1. The uncorrected lysine results are included for comparison. Reasonable agreement exists between the results obtained by the standardized and corrected TNBS method as we have developed it and a minor variation of the "blocked" lysine method of Blom et al. The low available lysine in roller dried whey compared to the others agrees with the nutritional deficiency of roller dried products (10).

TABLE 1. Available lysine determined by 2,4,6-trinitrobenzene sulfonic acid (TNBS) and the blocked method of four different whey powders.

Powder	Total lysine	Available lysine		
		Uncorrected TNBS	Corrected TNBS	Blocked method
	(g/100g Protein-total N × 6.38)			
Delactosed and spray dried	6.94	4.31	6.18	6.31
Lactalbumin	9.79	7.38	8.75	9.24
Spray dried	7.92	5.07	7.84	7.44
Roller dried	5.63	2.86	4.77	5.06

Discussion

It is possible to determine the relative amount of available lysine in whey and whey products by the TNBS procedure of Kakade and Liener provided that our corrections and modifications are employed. Our modified method is relatively fast and needs only simple equipment. Precision of the method is such that triplicate determinations should be run as suggested in the original paper. However, we found that it is also necessary to run triplicate blanks.

The variation in results with protein sample size is bothersome because, for whey products, it seems to be independent of the protein-reagent ratio. Handwerck et al. (7) suggested that in the case of fluorodinitrobenzene reagent, the epsilon-dinitrophenyl (DNP)-L-lysine derivative is destroyed due to reduction of the nitro groups during hydrolysis. Similar DNP-derivative destruction during analysis has been reported by Blom et al. Their suggested method to overcome this destruction by addition of dinitrophenol to the reaction mixture could not be duplicated in our work.

Our corrected TNBS data agree with results procured by determining available lysine as the difference between blocked and total lysine. In Table 1 the results with TNBS tend to be slightly low. However, when relative values are considered, our procedure gives valuable information about the composition of whey products.

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